

Taxonomic decision as a compromise: *Acasis appensata* (Eversmann, 1832) in Central Italy—a case of conflicting evidence between DNA barcode and morphology (Lepidoptera: Geometridae)

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Abstract

Acasis appensata (Eversmann, 1842) (Lepidoptera: Geometridae), distributed from central and northern Europe to eastern Asia, was collected in the Mediterranean (Central Italy, Abruzzi) for the first time. Adults from this region differ significantly from nominotypical populations in external appearance and in the 5' barcode fragment of the CO1 gene (barcode). However, morphology of male and female genitalia falls completely within the range of variation of *A. appensata*, resulting in conflicting evidence with respect to its taxonomic status. Considering the striking differences in genitalia morphology between the two European species of *Acasis*, viz. *A. appensata* and *A. viretata* (Hübner, 1799), the population from the Abruzzi is not validated as different at species level but described as the subspecies *A. appensata callaina* ssp. nov. The habitus and male and female genitalia of European taxa are figured.

Key words: Lepidoptera, Geometridae, *Acasis appensata*, morphology, DNA barcode, taxonomic rank, new subspecies, Europe, Italy

Introduction

Geometrid moths are a mega-diverse family of Lepidoptera with altogether about 23,000 described species (Scoble & Hausmann 2007) and numerous still undescribed taxa, mainly from tropical regions. The European fauna is particularly well known and the discovery of new species is the exception here. Such findings are usually confined to the Mediterranean region or to other exceptional areas such as the Alps (Huemer & Hausmann 2009). The genus *Acasis* dealt with in this paper has been considered well explored, and European species have been described already in the late 18th and the first half of the 19th century. In Europe *Acasis* comprises the widely distributed palae-arctic species *A. viretata* (Hübner, 1799) and *A. appensata* (Eversmann, 1842). Furthermore, the genus includes *Acasis bellaria* (Leech, 1891) and *A. exviretata* Inoue, 1982 from eastern Asia, and the nearctic *A. viridata* (Packard, 1873) (Scoble 1999). Species discrimination in Europe was hitherto considered unproblematic due to different external morphology of the adults and several specific characters of the genitalia of both sexes. Therefore it came as a surprise when during an excursion to the Abruzzi mountains in Central Italy the junior author and colleagues collected a series of specimens clearly belonging to *Acasis* but matching neither of the known European species. This doubtful population was already known to the senior author from two specimens collected in the same area a couple of years ago (H. Rietz pers. comm.) but material was insufficient for a taxonomic decision. Identification became increasingly complex when we started to evaluate diagnostic characters from phenotype, genitalia structures and DNA barcodes.

Material and methods

This study is based on about 700 dry and mostly set specimens belonging to the genus *Acasis*. Altogether we examined 3–6 genitalia slides of both sexes of all involved taxa. Genitalia preparation followed standardized techniques as described by Robinson (1976). Wingspan is defined as the distance between both forewing apices of set specimens. However, this measurement is sometimes difficult to take, particularly in females with rounded apices. Therefore the scale unit is only whole millimeters. Photographs of the adults were taken with an Olympus E3, Macro 50 mm 1:2. Genitalia were photographed with an Olympus E1 and an Olympus BH2 microscope. A total of 30 specimens of *Acasis* was processed at the Canadian Centre for DNA Barcoding (CCDB, Guelph) to obtain DNA barcodes using the standard high-throughput protocol as described by Ivanova *et al.* (2006). Altogether 28 specimens were successfully sequenced and analyzed with the database supplied by Barcode of Life Datasystems (BOLD). The data are accessible under the following GenBank Accession numbers: GU686755, HM376826, HM914421–914422, HQ601015–601017, HQ968307, HQ968351–968352, HQ968401–968402. Acronyms for institutional and private collections are as follows: RCTM—Research Collection Toni Mayr, Feldkirch Austria; TLMF—Tiroler Landesmuseum Ferdinandeum, Innsbruck, Austria; ZSM—Zoologische Staatssammlung, Munich, Germany; BIO—Biodiversity Institute of Ontario, University of Guelph, Canada.

Checklist of *Acasis*

Acasis Duponchel, 1845
 = *Agia* Hulst, 1896
 = *Bryodis* Gumpfenberg, 1887
 = *Cysteopteryx* Hulst, 1896
Acasis appensata (Eversmann, 1842)
Acasis appensata appensata (Eversmann, 1842)
 = *Lobophora appensata* var. *baikalensis* Bang-Haas, 1906
Acasis appensata callaina ssp. nov.
Acasis bellaria (Leech, 1891)
Acasis exviretata Inoue, 1892
Acasis viretata (Hübner, 1799)
Acasis viretata viretata (Hübner, 1799)
 = *Phalaena trinotata* Donovan, 1810
Acasis viretata himalayica Prout, 1958
Acasis viridata (Packard, 1873)
 = *Agia eborata* Hulst, 1896

Taxonomic results

Acasis appensata appensata (Eversmann, 1842)

Acidalia appensata Eversmann, 1842: 556.

Material examined. 233 specimens from Germany, Austria, Czech Republic, Slovakia, Byelorussia, Finland, Bulgaria, Greece, Turkey, Russia (TLMF; ZSM).

Description. Adult (Figs. 1–2). Head, thorax and tegula mixed grayish-brown, labial palpus dark brown. Wingspan ♂ 18–22 mm; ♀ 20–23 mm; forewing with strongly convex termen; ground colour grey-brown, extended darker grey-brown suffusion particularly at base and along antemedian and postmedian fascia; these two fasciae distinct, light greyish with indistinct darker line; veins with short dark brown lines along outer side of antemedial and both sides of postmedian fascia; dark brown discal spot and dark brown patches along fringe line well developed; fringes chequered greyish-brown, outer part lighter. Hindwing grey-brown with lighter postmedian fascia; small discal spot; darker grey terminal line and weakly chequered fringe; base of anal area in male with short

lobe. The extent of dark suffusion of the forewing underlies some variation. **Male genitalia** (Figs. 7–8). Uncus broad, sub-triangular, apically pointed; valva sub-oval, with rounded apex; sacculus strongly sclerotized, shorter than valva, basal part broad, distally tapering but rounded; costa with spiny sub-basal hump, cucullus separate, slender and slightly club-shaped, projecting far above apex of valva; saccus short, broad, horseshoe-shaped; lateral lobes of transtilla covered with short bristles; aedeagus moderately long and slender, weakly curved, without cornuti. **Female genitalia** (Fig. 15). Papillae anales well developed; apophyses posteriores about two times length of tergum A8; tergum A8 smoothly sclerotized, without modifications; apophyses anteriores comparatively long and rod-like, about half length of tergum A8; antrum (colliculum) tubular, posteriorly funnel-shaped, length (0.3–0.4 mm) about that of apophyses anteriores, sub-genital plate (sterigma) largely reduced; ductus bursae extremely short and without distinct transition to corpus bursae; corpus bursae evenly dilated to sub-oval distal part with a large spinulose patch in diffuse ring shaped position; appendix bursae present. Posterior part of corpus bursae with longitudinal furrows.

Diagnosis. *Acasis appensata appensata* is a rather invariable taxon with a grey-brown ground colour and distinctly lighter ante- and postmedian fasciae. The ground colour is of particular diagnostic value compared to the other European species *A. viretata* which exhibits an extended yellowish-green to brownish-green colour (Figs. 5–6), and to the pale green *A. appensata callaina* ssp. nov. (Figs. 3–4) (see below). *A. appensata* furthermore differs from *A. viretata* by several characters of the male and female genitalia (Figs. 13–14, 18). In the male the broad uncus, the long cucullus which overtops the apex of the valva, the spiny sub-basal hump of the costa, the short sacculus and the lateral lobes of the transtilla with short bristles are characteristic. In the female the long and narrow, funnel-shaped antrum, the largely reduced sub-genitalia plate and furthermore the anteriolateral margin of tergum VIII which is weakly projected anteriorly and the long apophysis anterioris are specific characters.

Bionomics. The larva of the nominotypical subspecies has been recorded feeding on unripe and also on ripe fruits which are loosely spun together, or on the leaves of *Actaea spicata* (Burmman 1955; Skou 1986), according to Skou (1986) also on *Veronica longifolia*. Feeding habits depend on the phenology of the host-plant which shows much variation, and on the geographic region (Burmman 1955). In northern Europe the species feeds on various other plants, e.g. *Valeriana sambucifolia*, *Actaea erythrocarpa* (Mutanen *et al.* 2003; Mutanen pers. comm.). The larval period lasts from mid-June to late July. Pupation takes place in weak cocoon on the ground surface and the pupa hibernates. Adults have been observed from mid-May until mid-June, in the northern part of the range throughout the entire month of June (Skou 1986). Whereas larvae are frequently found, the adults are rarely observed. Characteristic habitats are clearings in deciduous and mixed forests, particularly beech forest. The species occurs from lowland habitats up to about 1200 m above sea-level in the Alps.

Distribution. Vast range from Europe to the Urals, Siberia, Amur and Ussuri regions and Japan. In Europe occurring mainly in the Alps and adjacent areas and from Fennoscandia to central Urals. In northern Europe also on coastal meadows and deciduous forests and meadows along riversides (Mutanen *et al.* 2003; Mutanen pers. comm.). Absent from large parts of the Balkans, the Iberian Peninsula and from all Mediterranean lowlands and islands.

Remarks. The taxonomic status of the taxon *baikalensis* (original combination: *Lobophora appensata* v. *baikalensis*) needs further investigation. According to the original description it differs from Central European *A. appensata* by the dark blackish-grey ground colour and the on average smaller wingspan (Bang-Haas 1906). Following Scoble (1999) this taxon is considered as a synonym of nominotypical *A. appensata*.

Acasis appensata callaina Hausmann & Huemer ssp. nov.

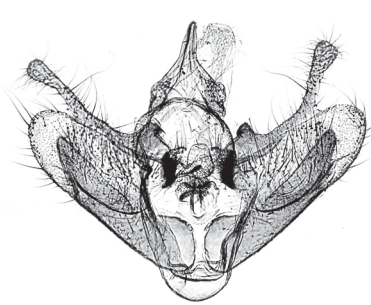
Type material. Holotype ♀, 'ITALIA, Abruzzan, Monte Terminillo, 1730 m, 13°00,6'E, 42°49,0'N, 16.07.2010, Huemer leg. TLMF 2010-015' (TLMF). Paratypes. Italy: 1 ♂, 10 ♀, same data as holotype (genitalia slides G 1456 ♀, G 1458 ♀, G 1461 ♂ (TLMF); 4 ♀, ditto, but 12.7.2010 (TLMF); 1 ♂, ditto, but 11.7.2010 (genitalia slide G 1464 ♂ (TLMF); 6 ♂, 6 ♀, ditto, but 11.7.2010, leg. Mayr (RCTM; coll. N. Pöll); 10 ♂, 16 ♀, ditto, but 12.7.2010, leg. Mayr (RCTM); 2 ♂, 12 ♀, ditto, but 16.7.2010, leg. Mayr (RCTM); 1 ♂, 6 ♀, ditto, but 17.7.2010, leg. Mayr (RCTM). 1 ♂, 1 ♀, ditto, but 1800 m, 15.7.2004, leg. H. Rietz (ZSM); 1 ♂, ditto, but 15.7.2007, leg. et coll. H. Rietz; 1 ♂, 2 ♀, ditto, but 17.-20.7.2004.



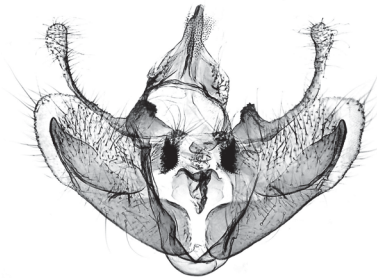
FIGURES 1–6. Adults of *Acasis*. 1–2, *A. appensata appensata* (Eversmann); 3–4, *A. appensata callaina* **ssp. nov.**; 5–6, *A. viretata* (Hübner).

Description. Adult (Figs. 3–4). Head, thorax and tegula mixed grayish-brown, labial palpus dark brown. Wingspan ♂ 20–21 mm; ♀ 20–23 mm; forewing with strongly convex outer margin; ground colour light greyish covered with pale green, darker grey suffusion particularly along undulated antemedian and postmedian fascia; postmedian fascia light greyish with indistinct darker line; veins with short dark brown lines along both sides of postmedian fascia; dark brown discal spot and dark brown patches along fringe line well developed; fringes chequered greyish-brown, outer part lighter. Hindwing grey-brown with slightly darker basal half; small discal spot; darker grey terminal line and weakly checkered fringes; base of anal area in male with short lobe. Male genitalia

(Figs. 9–12). As described under the nominotypical subspecies, but sacculus slightly more tapered towards apex.
Female genitalia (Figs. 16–17). As described under the nominotypical subspecies, but antrum (colliculum) shorter, length approx. 0.3 mm, posteriorly more clearly funnel-shaped (with deeper posterior excavation), posterior part of corpus bursae less furrowed.



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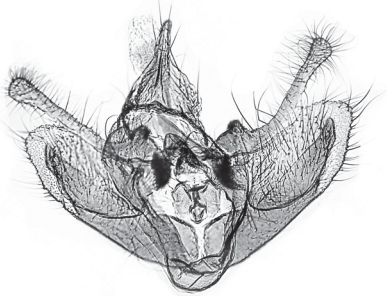
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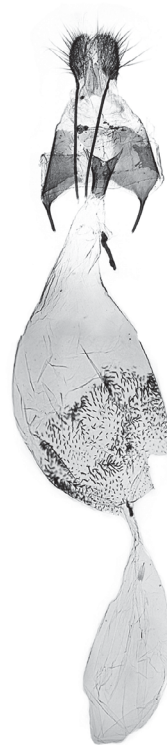


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FIGURES 7–14. Male genitalia of *Acasis*. 7–8, *A. appensata appensata* (Eversmann), slide G 1460; 9–10, *A. appensata callaina* **ssp. nov.**, paratype, slide G 1464; 11–12, *ditto*, paratype, slide G 1461; 13–14, *A. viretata* (Hübner), slide G 1462.



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17



18

FIGURES 15–18. Female genitalia of *Acasis*. 15, *A. appensata appensata* (Eversmann), slide G 1459; 16, *A. appensata callaina* **ssp. nov.**, paratype, slide G 1456; 17, *ditto*, paratype, slide G 1458; 18, *A. viretata* (Hübner), slide G 1463.



FIGURE 19. Type-locality of *A. appensata callaina* **ssp. nov.** (Monte Terminillo, Abruzzi, Italy).

Diagnosis. This new subspecies is characterized by the pale green suffusion of the forewings which is greyish-brown in the nominotypical subspecies (Figs. 1–2) and yellowish-green in *A. viretata* (Figs 5–6). The genitalia of both sexes are very similar to those of *A. appensata appensata* but strongly differ from those of *A. viretata* (see above and Figs. 7–18).

Bionomics. Host-plant and early stages are unknown but it is very likely that the new subspecies also feeds on *Actaea spicata* which occurs at the type-locality. The adults have been collected in mid-July nearby the upper edge of a beech forest on limestone at high altitudes of about 1700 m.s.l. (Fig. 19). They were attracted to artificial lights in large numbers.

Distribution. Only known from the northern slopes of Monte Terminillo in the Central Italian Abruzzi.

Etymology. The specific name, an adjective derived from the Latin *callainus*, refers to the pale green colour of the forewing.

Molecular data

Genetic distance (minimum pairwise distance, Kimura 2 parameter; see Table 1) is 2.83% between *Acasis appensata appensata* from Finland and Germany (n=6; maximum intraspecific distance 1.29%) and *Acasis appensata callaina* ssp.n. from central Italy (n=6; maximum intraspecific distance 0%). *Acasis appensata appensata* and *Acasis viretata* from Germany, Finland, Italy (n=9; maximum intraspecific distance 0.68%) diverge by 3.4%. The nearest neighbour of *Acasis appensata callaina* is the North American *Acasis viridata* (n=7) at a genetic distance of only 1.4%.

TABLE 1. Genetic distances (minimum pairwise distances, Kimura 2 parameter) between four examined taxa of the genus *Acasis* resulting from an analysis of full-length barcode fragments (COI 5', 658 bp).

	<i>A. viretata</i>	<i>A. a. appensata</i>	<i>A. a. callaina</i>
<i>A. a. appensata</i>	3.7%		
<i>A. a. callaina</i>	3.7%	2.8%	
<i>A. viridata</i>	3.1%	2.2%	1.4%

Discussion

European taxa of *Acasis* were hitherto considered unmistakable due to characteristic colour of the forewings combined with strong differences in genitalia morphology. Specific characters are furthermore reflected by larval host-plants and habits. *A. appensata* is probably restricted to *Actaea spicata* in Central Europe whereas *A. viretata* feeds on the flowers and fruits of usually woody plants such as *Frangula alnus*, *Viburnum opulus*, *Cornus sanguinea*, *Hedera helix*, *Acer pseudoplatanus*, *Ilex aquifolium* and *Sorbus aucuparia* (Bartsch *et al.* 2001).

Conflict 1: Genital morphology versus colouration

The discrimination of *A. appensata callaina* ssp. nov. from the nominotypical subspecies at first seems ambiguous. Obviously the central Italian population is isolated at a distance of about 500 km to the nearest reported occurrence of the *A. appensata* in Central Europe. Data about the bionomics are scarce and of limited value to define separate taxonomic status though the flight period of the adults is about 1-2 months later than in other populations. However, this peculiarity may be related to the elevation of the type-locality which exceeds alpine localities by about 500 m. Genitalia structures which are usually valuable for species discrimination in the genus *Acasis* are virtually indistinguishable between both subspecies. The only remaining character separating *A. appensata callaina* ssp. nov. is the pale green colour of the forewing. This character underlies no variation and doesn't overlap with the forewing colour in the nominotypical subspecies. Colour differences in Lepidoptera are frequently observed, particularly in regions with high edaphic variation. However, because of a character importance ranking which sets genital morphology higher than colouration and wing pattern, their taxonomic value is usually considered of minor importance though subspecies are frequently based on colour differences.

Conflict 2: Genital morphology versus genetic distance

Molecular data of the barcode region (CO1 gene 5') shine another light on the case of *A. appensata callaina* ssp. nov. Table 1 shows five genetic distances for the following species pairs: *A. viretata* and *A. appensata callaina* (3.7%), *A. viretata* and *A. appensata appensata* (3.7%), *A. viretata* and *A. viridata* (3.1%), *A. viridata* and *A. appensata appensata* (2.2%), *A. viridata* and *A. appensata callaina* (1.4%). These values average to 2.82% as typical species distances for the genus *Acasis*. In this light, the genetic distance of 2.83% between *A. appensata appensata* and *A. appensata callaina* suggests both taxa to be different at the species level. Because of zoogeographical reasons a conspecificity between the isolated Italian population of *A. appensata callaina* and the North American *A. viridata* seems unlikely, despite their lower divergence of just 1.4%. One examined and dissected male of *A. viridata* (BIO) showed its genitalia to be very similar to those *A. appensata*, the dorsobasal process of valva being slightly more projecting and bill-shaped, harpe narrower at tip and aedeagus stronger curved. Since these differences are very small, we believe that *A. viridata* may better be downgraded to subspecies rank of *A. appensata*. This should be tested in a subsequent study, basing on a larger sample of the North American taxon.

Conclusion: the barcode helps to justify the 'subspecies-compromise'

The 'conflict' between genital morphology of *A. appensata appensata* and *A. appensata callaina*, hardly justifying separation at species rank and the different colouration of both taxa requires a taxonomic decision, but cannot be resolved easily with these two data sets. So far, it was up to the subjective decisions of taxonomists to introduce (or not) subspecific names in such cases of allopatric populations with constant, characteristic colouration or wing pattern and taxonomy may have suffered from the description of too many 'substrate races' at formal subspecies

rank. Now, with DNA barcoding we have an additional data set to make such decisions more objective. In our case the molecular data suggest a long-term isolation with peculiar genetic differentiation. When considering all three data sets, genital morphology, colouration and DNA barcodes, the compromise of subspecies status for *callaina* is substantiated better than it was before.

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